Deterministic mathematical modelling for cancer chronotherapeutics: cell population dynamics and treatment optimisation

Jean Clairambault

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Abstract

In this short review paper, I will present the mathematical models that have been designed in the frame of continuous deterministic cell population dynamics that aim at optimisation of cancer treatments using chronotherapeutics. Many authors have dealt with chronobiology of cancer, less with continuous mathematical models and even less with the declared aim to optimise chronotherapeutics. The biological and theoretical bases for these models are sketched, started from a historical viewpoint, and the main theoretical results are presented, with biological suggestions to account for them. Chronotherapeutics, that leads to therapeutic optimisation with the contraint of limiting unwanted toxicity of anticancer drugs towards healthy cell populations, is put in a medical perspective together with the other main pitfall of cancer therapeutics, for which optimisation procedures should have little to do with circadian biology, i.e., emergence of drug resistance in cancer cell popuations, which is amenable to the use of other sorts of models, that are briefly mentioned.

1 Introduction

Chronotherapeutics has been designed and used for more than twenty years as an effective treatment against cancer by a few teams around the world, among whom one of the first is Francis Lévi's at Paul-Brousse hospital (Villejuif, France), in application of circadian clock physiology to determine best infusion times within the 24-hour span for anticancer drug delivery. Mathematical models have been called in the last ten years to give a rational basis to such optimised treatments, for use in

BANG project-team, INRIA Paris-Rocquencourt, BP 105, F78153 Rocquencourt, France jean.clairambault@inria.fr ·
and Lab. Jacques-Louis Lions, BC 187, UPMC, 4 Place Jussieu, F75252 Paris cedex 05, France

the laboratory and ultimately in the clinic. While actual clinical applications of the theoretical optimisation principles found have remained elusive so far to improve chronotherapeutic treatments in use, mathematical models provide proofs of concepts and tracks to be explored experimentally, to progress from theory to bedside.

Starting from a simple ordinary differential equation model that allowed setting and numerically solving a drug delivery optimisation problem with toxicity constraints, this modelling enterprise has been extended to represent the division cycle in proliferating cell populations with different molecular targets, to allow for the representation of anticancer drug combinations that are used in clinical oncology.

The main point to be made precise in such a therapeutic optimisation problem is to establish, here in the frame of circadian chronobiology, physiologically based differences between healthy and cancer cell populations in their responses to drugs. To this aim, clear biological evidence at the molecular level is still lacking, so that, starting from indirect observations at the experimental and clinical levels and from theoretical considerations on the model, speculations have been made, that will be exposed in this review of cancer chronotherapeutics models with the corresponding optimisation problems and their numerical solutions, to represent these differences between the two cell populations, with regard to circadian clock control.

2 Circadian clocks: biology and models

2.1 Short historical background

The existence of rhythms in natural phenomena, following a period that is grossly superimposable to the day-night alternation, for instance the folding and unfolding of leaves of plants, has been known since antiquity, and such rhythms had even been noticed by d'Ortous de Mairan in the XVIIIth century to occur also in constant darkness, thus being independent of the light of the Sun and hence intrinsically linked to some proper rhythm of the plants [98]. This was the beginning of *chronobiology*, the field of science that deals with biological rhythms. In particular in this case, a *circadian* rhythm, i.e., a rhythm with approximately 24-hour period had been evidenced in that plant (a Mimosa), but of course other rhythms of longer period, yearly (seasonal), monthly (menstrual) had been observed throughout the history of mankind. Circadian is a term that was coined in the late 1950s by the chronologist Halberg (1919-2013) on the basis of the latin *circa diem*, i.e., about a day, to qualify the period of a rhythm [61]; conventionally among chronobiologists, it means a rhythm with period between 20 and 28 hours.

Many circadian rhythms have been found in various organisms, fungi, plants, algae, insects, and more recently mammals, and the first gene known to be expressed according to a circadian rhythm was the *Per* gene, found in the fly *Drosophila melanogaster* in 1971 by Konopka and Benzer [70]. Then more and more genes expressed according to a circadian rhythm were found in cells of organisms that were

known to present such rhythmic phenomena (in particular the *Clock* gene in mice, by Takahashi in 1994 [103]), so that eventually the concept emerged of a *molecular circadian clock* constituted of genes and their resulting proteins in a network of activation and inhibition loops. Such a molecular circadian clock was found in mammals in every nucleated cell where it was searched for, and it was also shown all these cell clocks, though having their own periods, were under the control of a central circadian pacemaker located in the hypothalamus, the so-called suprachiasmatic nuclei (SCN), constituted of about 20000 neurons coupled together, giving rise to a common rhythm, itself reset by light through a retino-hypothalamic tract that normally ensures some synchrony between mammals of a same population [38, 52, 63].

Recognition of a circadian rhythm (period searched for between 20 and 28 hours) in a biological recording may be done by spectral analysis followed by cosinor analysis [84]. These are signal processing and statistical methods that do not explain any mechanism, but contribute to select biological variables as candidates to be regulated by circadian clocks. After identification of a possible period $T = \frac{2\pi}{\omega}$ in the recorded time series by spectral analysis, the mean, the amplitude, and the phase at maximum (acrophase) are determined according to the simple model $x(t_i) = M + A\cos\omega t_i \cos\varphi - A\sin\omega t_i \sin\varphi$ by least squares linear regression to determine $\alpha = M, \beta = A\cos\varphi$ and $\gamma = A\sin\varphi$ in $x(t_i) = \alpha + \beta y(t_i) + \gamma z(t_i)$, hence M, A and φ. Then a F-test is used to determine whether or not zero is in the confidence interval for the amplitude. If yes, the null hypothesis is not be rejected, and variations in amplitude of the signal are considered as non significant of an actual periodicity, but part of the background noise; conversely, if the null hypothesis is rejected, the time series is likely to present periodicity with period $T = \frac{2\pi}{\omega}$. Note that in order to accurately detect by spectral analysis a period T in a time series, a sample of length at least $2T$ must be available.

2.2 Modelling biological clocks

A simple way to design a biological clock, i.e., a periodic mechanism with molecular ingredients in nucleated cells is to use the following negative feedback loop: transcription (expression of a gene in the nucleus in the form of a messenger RNA) to translation (synthesis in the cytoplasm of a protein in a ribosome from its RNA), whence to inhibition of transcription (by a nuclear form of the translated protein, that goes from the cytoplasm into the nucleus, inhibiting its own transcription). Such a simple 3-variable ODE model of transcriptional regulation had been proposed as a general biological clock principle by Goodwin in 1965 [89], and later in 1999 by Didier Gonze, Jean-Christophe Leloup and Albert Goldbeter for the 24-hour rhythmic protein FRQ in the mould *Neurospora crassa* [73], after another more complex model for the protein PER in *Drosophila* had been proposed by Albert Goldbeter in 1995 [50]. Many other models of molecular circadian clocks have been published, including a very detailed one by Jean-Christophe Leloup and Albert Goldbeter in

2003 [72], all of them relying on activation and inhibition loops that had been evidenced by biological experiments.

These models are of single-cell oscillators, but it is possible to couple these oscillators, introducing some stochastic variability between cellular clocks, and study their synchronisation in the central circadian pacemaker in the SCN [14, 53, 54] and also to propose simple models of this central circadian control on independent peripheral cell clocks [23].

2.3 Influence of circadian rhythms on proliferation

At the individual cell level, clocks have been shown to influence both metabolism and proliferation for those cell population that are committed in the cell division cycle. As regards metabolism, the fact that some intracellular enzymes that process drug activation or detoxication show circadian behaviour in their gene expression or intracellular protein should be taken into account when representing time-dependent pharmacokinetics-pharmacodynamics (PK-PD) of anti-cancer drugs. One may recall here that pharmacokinetics describes by their concentrations the fate of drugs in the organism, from their infusion until their molecular target, while pharmacodynamics evaluates the actual effects of drugs on the organism, modifying its behaviour; in other words, according to a widely broadcast motto, "pharmacokinetics is what the body does to the drug, pharmacodynamics is what the drug does to the body". Independently of pharmacological actions, a indirect influence of circadian clocks on the cell division cycle has been evidenced, in particular by Georg Bjarnason in 1999 on the 24 hour-rhythm of the concentration of cell cycle determinants (Cyclins E and B1) in the oral mucosa of men [19], and also a direct one by Matsuo in 2003, showing that the circadian clock protein Bmal1 controls the G_2/M transition in the cell cycle through the kinase Wee1 [79]. Coupling between the circadian clock and the cell cycle has been modelled by Claude Gérard and Albert Goldbeter [47], based on this finding. Such representation of proliferation control by circadian clocks can also be used in cell population models of the cell cycle (i.e., not only at the individual cell level), as proposed in [23] using a FRQ-like model of the circadian clock, or also by using a simple cosine-like wave for the clock. A recent review on molecular mechanisms linking circadian clocks and the cell division cycle has been published in [78].

2.4 Differences between healthy and diseased clocks?

Peripheral circadian clocks are synchronised by the SCN [38, 52, 63] and such synchronisation may be experimentally disrupted, either by surgical ablation (thermocoagulation) or by out-of-phase non 24-hour periodic jet-lag like repeated entrainment by artificial light, in laboratory mice [63]. Both experimental conditions, re-

sulting in total loss of circadian rhythmicity in body temperature and rest-activity alternation, led to accelerated tumour progression in B6D2F1 mice, by comparison with a control group of the same strain in which normal entrainment by light on a physiological 24-hour basis (12 hours of light, 12 hours of darkness) was preserved [42, 43]. These experiments were led in Francis Lévi's laboratory at Paul-Brousse Hospital in Villejuif, France, a hospital in which at the same time are conducted treatments of metastatic colorectal cancer by a combination of cytotoxic drugs (5-Fluorouracil, Oxaliplatin, Irinotecan) delivered according to a circadian schedule [83] designed on a computer and implemented in a programmable pump. With 3-week autonomy, easily portable by the patients, valuing their quality of life, such pumps allow them to live and work normally. Francis Lévi and his clinical team [83] - but also others [94] - have observed that the more ablated physiological circadian rhythms are in patients - as evidenced by low amplitude of cortisol variations in blood, or of central temperature -, the poorer is their prognosis; otherwise said, a preserved physiological circadian rhythm is in favour of a good prognosis in patients with metastatic colorectal cancer under treatment. Additionally to external detrimental environmental factors such as shift work, that enhances the risk of developing several cancers [33, 62, 64, 92], a disrupted central SCN clock may be the result of the cancer disease itself, through circulating cytokines emitted in the tumour tissue by an immune reaction against it [87], and also by some anticancer drugs that have been shown to perturb the clock through a mechanism that is not known [74]. A general and recent review on circadian clocks and cancer, including cancer chronotherapeutics and proposed mechanisms to account for its efficiency, may be found in [44]

3 Using circadian chronobiology for cancer therapeutics

3.1 The case of cancer in therapeutics

Cancer is a disease of the physiological control on cell and tissue proliferation. In healthy organisms, normal regeneration of a tissue, based on the cell division cycle (at the term of which one cell becomes two) in renewing cell populations such as intestinal mucosa, haematopoietic bone marrow, skin and others, is physiologically controlled to ensure functional persistence of this tissue. For instance, the production of young red blood cells by the bone marrow compensates without excess the elimination of aging red blood cells in the spleen. In cancer, a defect of control results in overproduction of young cells and unlimited tumour tissue growth. In this respect, the present pharmacology of cancer occupies a special place in the treatment of diseases, since the cytotoxic drugs that constitute its core are not directed towards re-establishing normal physiological control (as is the case for instance with drugs used in cardiology, tending to ensure normal tissue perfusion by our cardiac pump), but are directed towards the elimination of tumour cells, as though these

were bacteria or parasites, whereas they are somatic cells endowed with the same basic genome as healthy cells, but in which proliferation control is impaired, usually due to a succession of mutations. Complementary treatments aiming at slowing down the growth of the tumour, either by choking its vascular environment (antiangiogenic agents [65]) or by antagonising growth factor receptors or blocking elements of intracellular signal transduction cascades downstream of them (monoclonal antibodies [93], tyrosine kinase inhibitors - TKIs - [76], the main weapons for the so-called targeted therapies) may be qualified cytostatic, as they are not used to kill cells (which is what cytotoxics are designed for) but only to slow down entrance or progression in the division cycle. Note however that at high doses cytostatic drugs may become cytotoxic. They are seldom used alone, and cannot control cancer proliferation by themselves.

Understanding where normal proliferation control is impaired is not easy and it is difficult to correct, hence the tough choice to try and eliminate all diseased cells by cytotoxic agents. Making use of non cell killing treatments by re-establishing physiological control of proliferation and letting diseased cells die as non adapted to a healthy organism environment would certainly be better, but these are seldom available. Only in very few cases, in particular chronic myelogenous leukemia (CML, see Section 3.2) and in acute promyelocytic leukemia (APL, a form of acute myeloblastic leukaemia, AML), have been identified mutations (in both these cases in fact chromosome translocations, giving rise to fusion genes BCR-Abl for CML and PML-RARa for APL), which yield chimeric proteins responsible for the disease, and these chimeric proteins can be eliminated by specific drugs, in particular Imatinib for BCR-Abl [41]. In APL, treatment by ATRA, a non-cell killing agent targeting the abnormal fusion protein responsible for the blockade of differentiation in myelopoiesis at the promyelocyte stage, - nevertheless consolidated by cytotoxic drugs, usually anthracyclines - results in the gradual elimination of diseased cells in the spleen and cure in more than 90% of cases, an exceptional feature in AML [60].

Only when such so-called ‘druggable targets’ (such as BCR-Abl or PML-RARa proteins) have been clearly identified as the only cause of the disease is it justified to represent the action of drugs at the single cell level; in all other cases, where more complex mechanisms underlying uncontrolled tissue proliferation are at work, the cell population level is the best one to describe and model the effects of drugs. Wherever modelling considerations may be lead to, such ‘targeted therapies’ are actively searched for in pharmaceutical research, with limited therapeutic success so far, either because most often, not just one, but numerous intracellular pathways are disrupted, or because of treatment complications (see Section 3.2).

Conversely, in a whole-body therapeutic perspective, others favour the idea of identifying and enhancing physiological controls on tissue proliferation, with the aim to use them in a preventive way, of course (diet, personal life hygiene, etc.), but also to some extent in therapeutics, taking advantage of their possible added action in present cancer treatments. The circadian system, constituted of the central SCN pacemaker and of all peripheral cell clocks, that receive synchronising messages from it, is one possible such control system on cell and tissue proliferation, acting by the known controls of G_2/M transition by Bmal1 through kinase Wee1 [79], and

also through the cyclin-dependent kinase inhibitor (CdkI) p27 [56] acting on G_1/S transition in the cell division cycle.

An essential component of the molecular circadian clock, the gene Per2, has been shown to be a tumour suppressor gene [44, 45], and the p53 protein, the so-called 'guardian of the genome', that controls both checkpoints, G_1/S and G_2/M, has also been shown to be controlled through its inhibitor Mdm2 by ATF4, a component of the circadian clock [66]. Circadian clocks thus exert their action on the cell division cycle by controlling gating (by cyclin-dependent kinases, Cdks) at cell cycles checkpoints, and this may be taken into account by scheduling drug infusion profiles that aim at preserving healthy cell populations, but they may also modulate the action of intracellular drug processing enzymes, and this may also be taken into account by time-scheduling of treatments. What is the most important effect of clocks in adapting to them a rational scheduling of drug delivery flows, by fitting them to gating at checkpoints in the cell division cycle or to enzymatic intracellular processing (in particular detoxication), is hard to decide, and there are experimental arguments towards taking both effects into account in models. The first choice necessarily involves modelling the cell division cycle divided into phases, while the second one implies modelling of molecular cell and tissue PK-PD for the drugs at stake.

3.2 Pitfalls encountered in cancer therapeutics

Since tissue proliferation is necessary to the maintenance of a multicellular organism, drugs that will limit cancer growth by inhibiting mechanisms of cell proliferation that are common to all fast renewing tissues (which is the case so far of most anticancer drugs) will also affect healthy tissue, limiting their use, so that finding and exploiting differences in behaviour towards these drugs between healthy and cancer cell populations is a major challenge of cancer therapeutics. In some rare cases where one isolated abnormal, disease-specific, molecule has been identified and may be inhibited by a drug, two conditions that are fulfilled in particular in the case of the chimeric protein BCR-Abl, responsible for CML, and that can be neutralised by specific drugs, the first of which was Imatinib [41], without being deleterious to healthy cells. This is the grail pursued by so-called targeted therapies, but very often, even if the target is reached, other unpredictable and unwanted targets are also reached in healthy cells, and hoped-for specificity is lost, which may result in withdrawal from the market (as was the case, for instance, for gemtuzumab-ozogamicin that had been proposed in the treatment of acute myelogenous leukemia and was withdrawn by its manufacturer in the US, due to unfavourable outcome in clinical trials, although the case is still an object of debates [88]). So that, even when a drug seems well targeted, unpredicted toxic side effects may not be excluded, that limit its use.

Even when a drug seems well targeted without major side effects, another phenomenon may occur in a cancer cell population, limiting or even leading to forsake

its use, which is the development of resistance to the drug at stake. Cancer cells, endowed with genomic instability, are often able to overexpress genes that make them able to develop mechanisms, such as drug efflux by ABC transporters [55], a phenomenon that may come with or without genetic mutations. In the former case, it involves selection of an established resistant cell clone at the expense of shrinking the other - drug-sensitive - cells in the tumour population by a Darwinian mechanism, and in the latter, it is due to epigenetic modifications (modifications in genes that control expression of genes coding for proteins) that are reversible, i.e., not irreversibly inscribed in the genome. In either case, the right level to describe this drug-limiting phenomenon is the population of cancer cells, in which biological (phenotypic, even though the population may be genetically homogeneous [48]) variability can be taken into account.

These two obstacles, unwanted toxicity to healthy cells and the development of resistance to the treatment in cancer cells, are the two major pitfalls encountered in cancer therapeutics and they define the major constraints to be fulfilled by anticancer treatments: to avoid toxicity to healthy cell populations and to avoid emergence of drug resistance in cancer cell populations.

3.3 An optimisation problem under constraints

From these considerations results a conception of cancer therapeutics, using cytotoxic and cytostatic drugs, as an optimisation problem, where the objective is to contain tumour cell populations within limits compatible with the patients' life and a good quality of life (rather than eradication of tumour cells, an objective less easy to reach), under the constraints to preserve healthy cell populations - according to preservation criteria that have to be appreciated by the physician according to his patient's state of health - and to avoid the development of an uncontrollable drug resistant tumour cell clone. The most difficult elements to define in such a perspective of modelling proliferation in cell populations towards therapeutic optimisation are clear differences between healthy and cancer cell populations, and between sensitive and resistant cancer cell populations. As regards contrasts between healthy and tumour cell populations, it has been proposed to characterise them according to the behaviour of the two cell populations with respect to circadian control but so far, only assumptions without biological certainty on mechanisms can be made. Nevertheless, such assumptions allow to draw proofs of concepts by modelling, allowing to numerically solve optimisation problems much ahead of validation of these assumed biological characterisations.

In the sequel, I will propose modelling frames that have been used for these cell populations and for physiological and pharmacological control on their proliferation. Most of the work that has been done in this direction was performed in conjunction with Francis Lévi's team and is related to chronotherapeutics, directed towards solving the toxicity constraint problem; but I will also ultimately show more

recent results, out of the chronotherapeutic framework (that is not necessarily relevant in this case), in which the drug resistance constraint is taken into account.

4 Drug delivery optimisation and chronotherapeutics

4.1 Molecular pharmacokinetics-pharmacodynamics

To represent the action of a drug, delivered in the general circulation (this includes oral route, through an absorption mechanism that is usually intestinal and hepatic, but most often it is processed by direct intravenous infusion), on its target, wanted (therapeutic efficacy) or unwanted (toxic side effects), one must represent its fate in the organism from its infusion to its effects by PK-PD models. PK-PD depends on the drug at stake and is usually represented by a system of ODEs for concentrations of the different compounds. The parameters of these ODEs depend on the organism under study, and for some of them, e.g., kinetic constants of drug detoxication enzymes, on circadian rhythms within this organism; in the perspective of personalised medicine, they should ideally be identified in each patient to propose actually individualised treatments.

In the frame of chronobiology, the patient population level, that is the classic one in clinical PK-PD is not at the forefront (although, in a pioneering study, chronotoxicity of anticancer drugs has been tested in different mice strains [7]); rather, in molecular PK-PD for chronotherapeutics, a single organism with its different organs and cell populations concerned by the fate of the drug at stake is the object of study. Whole Body Physiologically Based PK-PD (WBPBPKPD, a term coined by Malcolm Rowland [101]) based on ODEs and exemplified (without circadian clocks) for 5-fluorouracil by Tsukamoto *et al.* [102] is an aim to be pursued, with the addition of circadian influences on drug processing mechanisms when relevant.

4.2 A simple ODE model based on a simplifying assumption

With the aim to simultaneously represent the dynamics of a cancer cell population, therapeutic target, and of a healthy cell population, unwanted toxicity target of the same anticancer drug delivery to be subsequently optimised, a simple ODE model has been designed and partly identified on tumour growth curves in mice from Francis Lévi's lab, with and without treatment by Oxaliplatin [8, 22]. Focus was put on *chronopharmacodynamics* to define differences between healthy and cancer cell population behaviour in response to the treatment: it had been experimentally observed at the lab that injection schedules (oxaliplatin was delivered in boli at fixed times of the 24-hour span) that led to more therapeutic efficacy (as measured by decrease in tumour growth curves) were at the same time those leading to least toxicity

(as measured by total body weight loss). Hence this simple modelling assumption: the hour of best therapeutic efficacy should coincide with the hour of least toxicity, i.e., pharmacodynamic effects should be phase-opposed between the two cell populations.

This is of course a very simplified assumption, in favour of which no known biological mechanism exists. It relies only on macroscopic observations and these observations are made according to a rather poor sampling frequency of injections, 6 in the 24-hour span, i.e., a 4-hour time resolution. Nevertheless, establishing a clear difference in behaviour between the two cell populations with respect to their responses to the drug infusion, it allowed to put in practice an optimisation algorithm for a continuous drug delivery schedule under toxicity constraint, yielding at least a proof of concept for this optimisation strategy, provided that actual differences with respect to circadian influence between healthy and cancer cell populations exist. The system of ODEs runs as follows

A damped harmonic oscillator stands for healthy cell population dynamics:

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V_{dist}}\Phi(t) \tag{1}$$

$$\frac{dC}{dt} = -\mu C + \xi_C P \tag{2}$$

$$\frac{dZ}{dt} = \{-\alpha - f(C,t)\}\, Z - \beta A + \gamma \tag{3}$$

$$\frac{dA}{dt} = Z - Z_{eq}, \tag{4}$$

where

$$f(C,t) = F\left(1 + \cos(2\pi\frac{t-\varphi_A}{24})\right)\frac{C^{\gamma_A}}{C_{50}^{\gamma_A} + C^{\gamma_A}},$$

and $\lambda, \mu, \xi_C, \alpha, \beta, \gamma, Z_{eq}, F, \varphi_A, \gamma_A, C_{50}$ are positive constants, identified on tumour growth curves or from literature data [22], or else estimated.. These equations represent drug diffusion and elimination by first order pharmacokinetics for concentrations in the plasmatic and target cell compartments (P and C), from infusion in the general circulation according to the instantaneous drug delivery flow $i(t)$ (Φ representing a "tap on-tap off" function), and healthy tissue (normal jejunal mucosa, here) homeostasis by a linear system showing a stable focus at $(Z_{eq}, A_{eq} = \beta^{-1}(\gamma - \alpha Z_{eq}))$, perturbed by the drug toxicity function which comes to strengthen the natural autoregulation coefficient α.

A Gompertz model stands for tumour cell population dynamics:

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V_{dist}}\Phi(t) \tag{5}$$

$$\frac{dD}{dt} = -\nu D + \xi_D P \tag{6}$$

$$\frac{dB}{dt} = -aB\ln(\frac{B}{B_{max}}) - g(D,t)B \tag{7}$$

Note that equations (1) and (5) are exactly the same, since they both represent the distribution of the drug in the plasma after infusion, and this the only feature these two systems, representing two cell populations have in common, since they are physically apart from each other: experimentally the tumour, a Glasgow osteosarcoma was implemented under the skin, whereas the main toxicity target in this mouse population was identified to be the jejunal mucosa. In this system of equations, function g, which represents anti-tumour drug efficacy, is assumed, as is function f for toxicity, to present circadian variations; it is given by:

$$g(D,t) = H\left(1+\cos(2\pi\frac{t-\varphi_B}{24})\right)\frac{D^{\gamma_B}}{D_{50}^{\gamma_B}+D^{\gamma_B}},$$

and $\lambda, \nu, \xi_D, a, B_{max}, H, \varphi_B, \gamma_B, D_{50}$ are positive constants, identified on tumour growth curves or from literature data [22], or else estimated. The difference of behaviours between the two populations of cells with respect to drug response is coded as $\varphi_A - \varphi_B = 12$ hours.

4.3 Numerical optimisation of drug delivery

Using this simple system of ODEs, it was possible to tackle the problem of drug delivery optimisation, i.e., minimisation of the tumour cell population under the constraint of minimising unwanted toxicity on the healthy cell population by keeping it under a prescribed level (to be in future clinical applications defined by the clinician in charge), by a nonlinear conjugate gradient method [8]. Note that this method consists of numerical optimisation, and it does not yield an optimal solution, but rather a suboptimal solution (the algorithm searches saddle points of a Lagrangian, and since the problem is not convex, it yields only necessary, not sufficient conditions of optimality), so that one could not completely exclude the existence of local minima in the descent algorithm yielding the best infusion profile [8]. Nevertheless, the existence of a global minimum can be proved, assuming for the evolution of the two cell populations $A(t)$ and $B(t)$ reasonable differentiability conditions with respect to time [8], which amounts to numerically solve a problem for which we know a unique solution to exist. Furthermore, the optimisation problem may be set in at least two different forms: the eradication problem consists in minimising the minimum of tumor cells, whereas the stabilisation (tumour containment) problem consists in minimising the maximum of tumour cells in a given observation window. Figure 1 shows the results of such a stabilisation procedure.

However, this optimisation procedure has two main flaws: it opposes the behaviours of the tumour cell population and of the healthy cell population by an assumption which is far from granted in general (a 12-hour dephasing between their maximal sensitivity to the drug), and it represents the action of a single drug on a single target (a death rate), which excludes the representation of combinations of drugs acting on different biological targets. But in clinical settings, most anticancer treat-

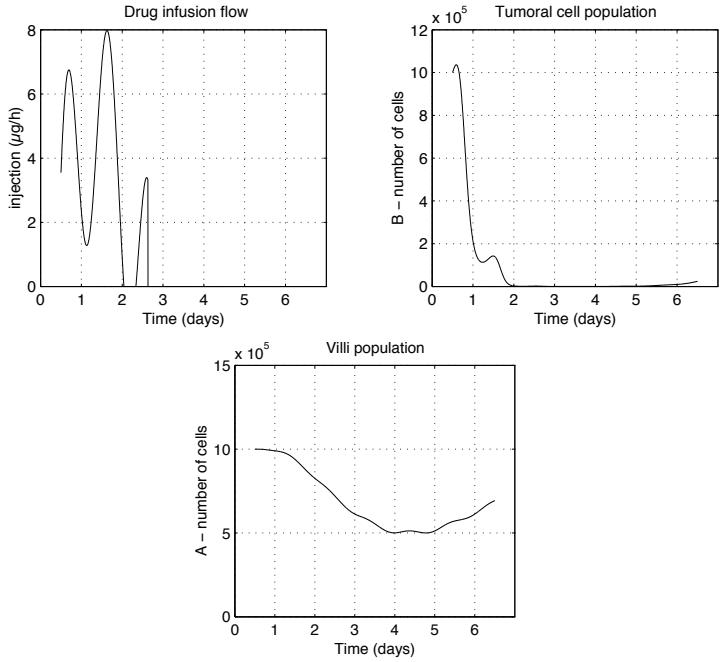

Fig. 1 Quasi-optimal solution of the stabilisation (tumor containment by repeated drug delivery courses) problem under absolute constraint of preserving at least 50% of the equilibrium population of healthy cells, for a duration of 2 days in a 7-day observation window. The optimal drug infusion flow i (left, upper panel) shows peaks at the times of minimal unwanted toxicity that are assumed to be simultaneously those of maximum therapeutic efficacy. The resulting tumour cell population (right, upper panel) is contained - treatment courses being repeated -, but not eradicated, while the healthy cell population (lower panel) is preserved over a prescribed level, here 50% of the equilibrium population. See Ref. [8] for details.

ments combine different drugs, all of them resulting in blocking or slowing down the cell division cycle on which relies all tissue proliferation, healthy or tumour, but acting on different molecular targets to potentiate their combined effects on cancer cell populations: cytotoxic drugs hit the DNA or cell proteins that are essential to cell division, leading cells committed in the division cycle to their inevitable death, while cytostatic drugs only slow down the division cycle, at least at non-massive doses. Hence the necessity to re-examine the ways by which differences between healthy and tumour cell populations should be represented with respect to their responses to drug treatments, and to design a model of the cell division cycle amenable to represent *at the cell population level* the different molecular targets of the various anticancer drugs that are in use in the clinic.

5 Cell cycle modelling using PDEs in cell populations

5.1 An age-structured McKendrick model with periodic control

The so-called McKendrick, or Von Foerster-McKendrick model of growing population dynamics was introduced in an integral form in 1911 by Sharpe and Lotka [95] in demography, and then independently and under its PDE form in 1926 by McKendrick [81], to be rediscovered in 1959 by Von Foerster [104]. It has been studied in detail, e.g. in [6, 67, 82]. Applied to the cell division cycle represented as an age-structured population dynamics model organised in a merry-go-round of subpopulations biologically identified as phases (G_1, S, G_2 and M), it was first proposed in 2003 in [28] under the form

$$\begin{cases} \dfrac{\partial n_i(t,x)}{\partial t} + \dfrac{\partial n_i(t,x)}{\partial x} + d_i(t,x)n_i(t,x) + K_{i\to i+1}(t,x)n_i(t,x) = 0, \\ n_{i+1}(t,0) = \int_0^\infty K_{i\to i+1}(t,x)n_i(t,x)dx, \\ n_1(t,0) = 2\int_0^\infty K_{I\to 1}(t,x)n_I(t,x)dx, \end{cases}$$

together with initial conditions $(n_i(t=0,.))_{1\le i\le I}$. Death rates in phases are noted d_i and transition rates between phases, assumed to be time-periodic, $K_{i\to i+1}$. Phase i ($1 \le i \le I$) may be one of the classical four G_1, S, G_2 and M, but also an aggregated phase such as $S - G_2$, or even a single proliferating phase $G_1 - S - G_2 - M$, or on the contrary a subdivision inside a phase, e.g., pre- or post-restriction point in G_1; the equation describes the evolution of the densities $n_i(t,x)$ of cells having age x at time t in phase i.

Let me stress here that age x is a 'physiological but abstract' variable, that lumps together complex unidirectional (in time) biological phenomena occurring in the cell machinery, that are based on protein synthesis to achieve cell division. Variable x has nothing to do with spatial distribution of cells in their population (space is considered here as irrelevant), since $n_i(t,x)$ is a density of cells that are at universal time t at a stage x of their way in phase i (according to an abstract clock measuring the degree of proteic synthesis), starting from 0 to transit to next phase without fixed time limit, but governed by the transition rate function $K_{i\to i+1}$. The proteic synthesis-related clock that governs evolution within G_1 and G_2 phases (preparing, respectively, S and M phases) might theoretically be followed by the concentrations of Cyclin D and A, respectively, but this is not the way it has been done so far (see below Section 5.2 about the FUCCI analysis method). However, phase S may be readily followed in flow cytometry by the synthesis of DNA, from 2n to 4n chromosomes, a method that has been used by Britta Basse and colleagues [9, 10], giving an immediate interpretation of age x in this case.

Such space-independent representation of the distribution of cells in their population is particularly adapted to taking into account proliferation control by drugs assumed to be homogeneously distributed in concentration in the cell population.

Taking spatial distribution of drugs into account should lead to more complex models, structured in both space and a physiological variable like age, but this would show useful only when a spatial distribution of cells in the tumour is known, and apart from the case of (very small) avascular tumours organised in spheroids, the topology of tumours is seldom known. *Mutatis mutandis*, the idea of representing tumour growth by an age-structured, rather than spatial, model when the cell division cycle, target of anticancer drugs by different molecular mechanisms, is the most relevant feature to be taken into account, is of the same order as in integration theory choosing the Lebesgue than the Riemann integral: it is just more practical.

The main output of such a linear model is its first eigenvalue λ, the so-called Perron eigenvalue, which, assuming minimal hypotheses on the parameters of the model, is always positive and simple (i.e., the associated eigenspace is generated by a single function, that may be normalised to be of unit integral, hence bounded). Moreover, it may be shown, using the Krein-Rutman theorem (an infinite-dimensional version of the Perron-Fröbenius theorem) that in each phase the solution converges for large times, in a L^1 sense, to $e^{\lambda t}$ times a fixed multiple of the associated normalised eigenvector [86], i.e., the behaviour of the solutions in all phases is governed by an exponential term given by the Perron eigenvalue, which of course is nothing but $\ln 2$ divided by the doubling time of the population. This means that knowledge of the death rates and of transition rates, targets of internal physiological or external pharmacological control entirely determine the proliferative behaviour of the population by its first eigenvalue λ, which is thus the main predictive output of the model.

In the case where transition rates $K_{i\to i+1}$ are time-independent, it is easy to see that the function of age x in phase i

$$f_i : x \mapsto K_{i\to i+1}(x)e^{-\int_0^x K_{i\to i+1}(\xi)d\xi}$$

is the probability density function (p.d.f.) of the duration of this phase, and it has been proved that for a given family of p.d.f.s with varying variance, the first eigenvalue λ increases with increasing variance of their p.d.f.s (one phase is enough) [18]. This result has the simple following interpretation: the more variable the duration of phases (i.e. the overlapping between phases), the faster the cell population proliferation. This may be put in relation with the observations of Section 2.4, at least if one admits that a disruption of circadian control on phase transitions should result in enhanced variability of the duration of phases (and hence of overlapping between them). However, this mathematical result has been proved only in the case $K_{i\to i+1} = K_{i\to i+1}(x)$, and not in the case when $K_{i\to i+1}$ is also time-dependent.

In [25, 26, 27, 29], the question of the influence of a periodic control - circadian, i.e., physiological, or pharmacological, i.e., external - has been examined. This question arose from the biological observations mentioned in Section 2.4, reported in [42, 43], since circadian control is by definition time-periodic. This induced to compare in the McKendrick model the evolution - as measured by its first eigenvalue - of a periodically controlled (on transition or death rates) proliferating cell population model with the version of the same model where periodic functions are replaced by

their arithmetic time averages (i.e., no more periodic control). By analogy with the above mentioned biological observations, the authors of Ref. [27] expected the first eigenvalue of the former to be lesser than those of the latter. To their surprise, they proved (Theorem 1 in [27]) that the opposite is true, i.e., periodic control enhances proliferation, at least if the control is exerted only on death rates, and that if one uses an arithmetico-geometric form of the time average (difficult, however, to justify biologically), this result holds true also for transition rates [25, 26], but that without this use of an arithmetico-geometric mean, it is impossible in general to predict how a time-periodic control on the transition rates will affect cell population growth.

These results may be interpreted, in the light of the observations mentioned in Section 2.4 in at least two different ways:

Firstly, if one admits that the use of the arithmetico-geometric mean is correct, it might be that only healthy tissues (tumour-surrounding and immune cells) committed in fighting the tumour development are sensitive to the messages of the central circadian clock pacemaker that is disrupted (surgically ablated [43] or perturbed by chronic jet-lag-like light entrainment [42]) and are thus weakened in their proliferation by the disruptions of the clock, whereas tumour tissues, relatively insensitive to circadian commands, proliferate unabashed and with less opposition from tumour-combating healthy tissues.

Secondly, if one leaves aside the use of the arithmetico-geometrical mean and if one admits that tumour cell populations are somehow sensitive to circadian commands, it may mean that circadian messages are not, or little, exerted on death rates, but only on transition rates. It is biologically known, in fact, that clock-controlled genes exert their influences, via Cyclin-Cdk complexes that control G_1/S and G_2/M transitions, as mentioned in Section 3.1, on transitions between phases (let us recall here that Cdk is abridged from cyclin-dependent kinase; the most important Cdks in the cell cycle are Cdk1, that needs Cyclin B to be activated and let cells process from phase G_2 to phase M, and Cdk2, that needs Cyclin E to be activated and let cells process from phase G_1 to phase S).

If the second interpretation is true, it only means that it is useless to look for circadian control on the apoptotic cascade. If the first one is true, it means that taking into account the influence of circadian inputs on healthy cell populations combating tumour development should be represented in a competition model, which remains to be done. Both interpretations may be right, recalling however, as mentioned in Section 3.1, that indirect circadian influences on death rates should exist, via circadian control on the activity of some intracellular drug processing enzymes (activation or degradation) and also via circadian control on Mdm2 [66], the main inhibitor of protein p53.

5.2 Another optimisation problem under toxicity constraints

Now, what is the biological reality of the McKendrick model and how can it be experimentally identified? To answer this question, observation of proliferating cell

populations was needed. This was made possible, thanks to a newly released analysis technique coming in 2008 from Miyawaki's lab in Japan, the so-called FUCCI (Fluorescence Ubiquitination-based Cell Cycle Indicator) analysis method, which made possible recording individual living and proliferating cells in a culture medium [90, 91] in two phases of the cell cycle: G_1 and $S-G_2-M$, and also thanks to the European consortium C5Sys (2010-2013), in which such measurements were performed in cultures of proliferating NIH3T3 cells. The FUCCI technology allows to follow individual proliferating cells and to measure the durations of the G_1 phase, and of the complete cycle, using prior hybridisation with fluorescent proteins of physiological proteins characteristic of G_1 or of $S-G_2-M$ phase.

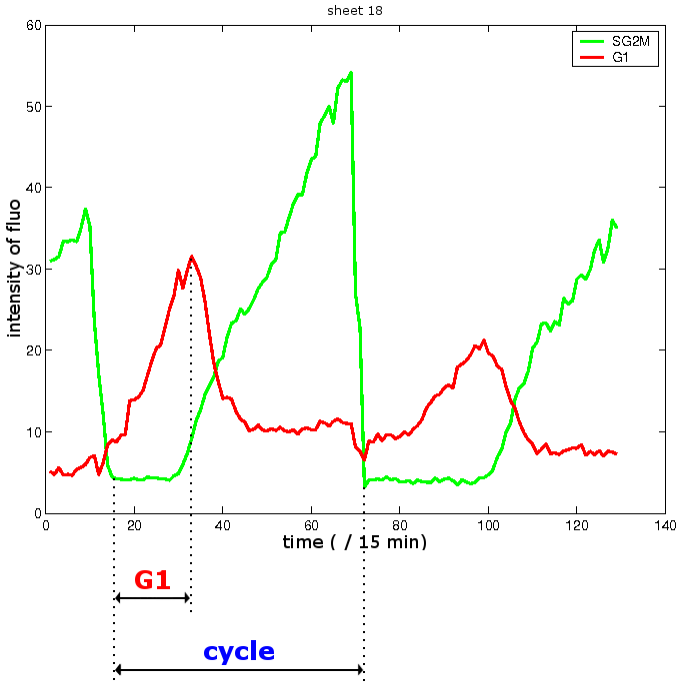

Fig. 2 After [17], method to determine the duration of phases G_1 and $S-G_2-M$, on FUCCI recordings [90, 91], on one cell committed in the division cycle. See References [17, 18] for details.

What simplifies the identification of the McKendrick model in this case is that cells that were followed during their division cycle (most often only one cycle was observed) were by definition living cells from the beginning to the end of the recording, i.e., death terms were nil, and that these NIH3T3 cells were moving freely in a liquid medium, with no communication between them, nor with any external influence applied. This means that transition rates $K_{i\to i+1}$ in the cell population were completely time-independent, representing only the biological variability of the cell

population with respect to age x in each of the two phases. Under these conditions, recalling that

$$f_i(x) = K_{i\to i+1}(x)e^{-\int_0^x K_{i\to i+1}(\xi)d\xi}$$

is the p.d.f. of the duration of phase i, which may be experimentally evaluated using FUCCI recordings of individual cells in the population [18, 16], straightforward computation yields the inversion formula

$$K_{i\to i+1}(x) = \frac{f_i(x)}{1-\int_0^x f_i(\xi)d\xi},$$

so that in the case of this NIH3T3 cell population in culture, the 2-phase McKendrick model is completely identified.

Assuming then that the periodic controls on the cell division cycle, both circadian (built-in) and pharmacological (tunable), are exerted on transition rates only, we had to hypothesize (or, better, to experimentally identify, which unfortunately did not prove possible with the FUCCI data provided in the C5Sys consortium) clear differences between healthy and cancer populations, in order to tackle in these new modelling settings the same optimisation problem as in Section 4.3: maximising tumour cell kill under the constraint of preserving a healthy cell population. Taking the model identified on NIH3T3 cells as a likely basis for a generic proliferating cell population, we hypothesized that such differences were due only to a difference in the effects of circadian messages on gating by Cyclin-Cdk complexes at phase transitions: sharp gating in the healthy case, resulting in small overlapping between phases, and loose gating in the tumour case, resulting in much broader overlapping, as sketched on Fig. 3.

These theoretical gating functions (hereafter noted ψ_i) occur in the model as time-dependent multiplicative modulating factors for the $K_{i\to i+1}(x)$, These gating functions thus synchronise cells with respect to cell cycle timing in cell populations, that are well synchronised when the gating is sharp (gate open during a brief interval of time), and poorly synchronised when it is loose. This modelling choice relies on the intuitive, not proven, but likely assumption (private conversation with F. Lévi) that healthy cell populations are more synchronised than cancer cell populations with respect to cell cycle timing, and that such synchronisation is due to the central circadian clock, i.e., the circadian pacemaker makes healthy cells pass in a 'disciplined' and orderly way from one phase to the next, while cancer cells, less 'obedient' to messages of the clock, pass in a disorderly way.

In this model setting, the transition functions without control, representing only the biological variability with respect to phase durations in age x within the cell population are chosen as the functions $\kappa_{i\to i+1}(x)$ identified on the NIH3T3 cell population, the circadian influence on gating is represented by the fixed functions $\psi_i(t)$ sketched on Figure 3, and if $g(t)$ ($0 \leq g(t) \leq 1$) is the drug infusion flow to be optimised, blocking cell cycle transitions, the complete transition rates are defined in the model as $K_{i\to i+1}(x,t) = [1-g(t)].\psi_i(t).\kappa_{i\to i+1}(x)$ ($1 \leq i \leq 2$), see on Fig. 3 the illustration of the corrected gating function $[1-g(t)].\psi_i(t)$ (dashed curve). As in Section 4.3, the optimisation algorithm searches for the function g (in fact, a

bang-bang one), except that in this case the observed outputs are not cell population numbers, but first eigenvalues representing proliferation rates (see Fig. 4): the first eigenvalue of the cancer cell population is minimised while maintaining the first eigenvalue of the healthy tissue over a prescribed fixed threshold (to be defined in future clinical applications by the clinician in charge), a situation comparable with the optimisation problem of Section 4.3, where the healthy cell population number had to be maintained over a prescribed fixed percentage of its equilibrium level. In a different model setting, this is another proof of concept for the optimisation method.

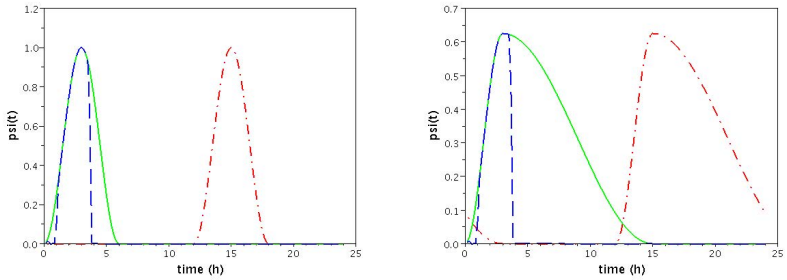

Fig. 3 Gating functions $\psi_i(t)$ at 2 phase transitions representing as functions of time the theoretical activity of the two Cyclin-Cdk complexes (for G_1/S and G_2/M) under circadian control, with a dephasing set at 12 hours between phase transitions (12 hours numerically yielding highest proliferation rates). When gating functions are nil, there is no transition between phases. Here is represented the assumed difference between healthy and cancer cell populations: sharp gating (left panel) for healthy tissues, loose gating (right panel) for tumours. The dashed curve common to both panels represents the corrected (by treatment) gating function $[1 - g(t)].\psi_2(t)$, where g is the output of the algorithm, solution to the optimisation problem, prescribing to deliver a cancer drug (by the general circulation to both tissues simultaneously) so as to result locally (at the tumour and at the healthy tissue site) in the pharmacodynamic function g. See text and Ref. [17, 18] for details.

An illustration of this method is presented on Figure 3. It represents (dashed curve) the optimised delivery of a drug that is active on transition G_2/M only, e.g., 5-Fluorouracil. Note that the PK of such a drug, from infusion in the general circulation until its presence on the target tissue site (tumour or healthy tissue) is not represented here. It should be added to the model equations to allow for accurate optimization of an actual intravenous infusion flow. Note also that whereas the FUCCI recordings should give us access to the M/G_1 transition, we rather assume that the duration of phase M is fixed, all cells in M passing into G_1 in fixed time (about one hour), and that the variability in age duration of the aggregated phase $S - G_2 - M$ is in fact that of $S - G_2$, so that the p.d.f. $f_2(x)$ gives us access by the inversion formula, under this assumption, to a $K_{2\to1}(x)$ transition function that is thus in fact related to the G_2/M transition.

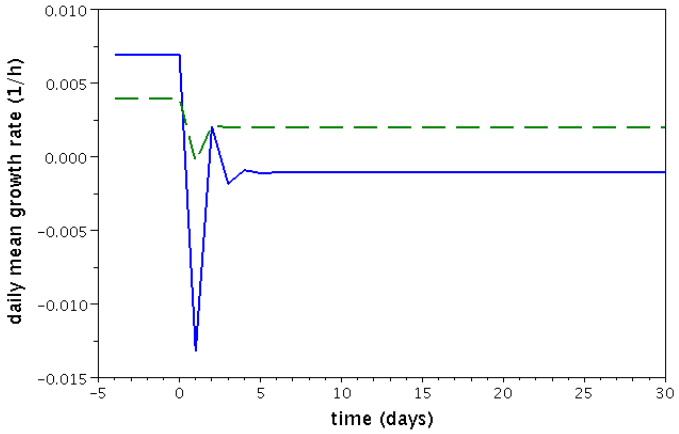

Fig. 4 Results of the optimisation method, from [17]. Contrary to the model presented in Section 4.3, the outputs are not cell population numbers, but proliferation rates. On can see that after a short transient time interval, the proliferation rate of the healthy cell population, initially disadvantaged, overcomes the tumour proliferation rate. See text and Ref. [17, 18] for details.

5.3 Possible extensions of or alternatives to the McKendrick model

One possible nonlinear extension consists in introducing exchanges (in both directions) between the proliferating population, divided or not in phases, and a quiescent population seen as a storage tank of cells that may be recruited in the proliferation cycle when needed, and to which an overflow of proliferating cells may be discharged when they lack energy resources, as introduced by Mats Gyllenberg and Glenn Webb [39, 58, 59] to give a mechanistic explanation to the Gompertz growth curves often encountered in population dynamics, and in a series of articles following [12] ([11, 20, 21, 40]). Note that in a model with several proliferative phases, the exchanges should be located in G_1 before the restriction point, and that the input of cytostatic drugs could be represented as slowing down recruitment into proliferation or enhancing way out to quiescence. Such models give rise to biologically realistic situations representing space and nutrient limitations, and a clear difference, relying on the recruitment function from the quiescence phase, may be set between healthy and cancer cell populations [11, 12]. But this difference has no relation with circadian clocks, which could be introduced, as in Section 5.1 by their action on phase transitions; this remains a open modelling problem. Note that if the model is no longer linear, one cannot speak of eigenvalues any more; however, linearisations around particular points of the population numbers may be studied [11, 12].

Another simple way to represent exchanges with a quiescent population in a still linear model is to exclude feedback from quiescence to proliferation, considering quiescence only as a sideway expansion cell tank, as proposed in [46]:

$$\begin{cases} \dfrac{\partial}{\partial t}p(t,x) + \dfrac{\partial}{\partial x}p(t,x) + \{\mu + K(x)\}\ p(t,x) = 0\ \ , \\ p(t,x=0) = 2(1-f)\displaystyle\int_{\xi\geq 0} K(\xi)\ p(t,\xi)\ d\xi\ \ , \\ p(t,x=0) = p_0(x)\ \ , \\ \dfrac{d}{dt}Q(t) = 2f\displaystyle\int_{\xi\geq 0} K(\xi)\ p(t,\xi)\ d\xi - \nu Q(t)\ \ , \\ Q(0) = Q_0\ \ . \end{cases}$$

In this age-structured McKendrick model designed to theoretically study the action of a cytostatic drug enhancing the way out of proliferating cells with density $p(t,x)$ to quiescent cells with density $Q(t)$, the drug target here is f, rate of escape at mitosis towards the siding phase Q, f to be enhanced by a cytostatic drug. The model [46] (see also [15]) was identified on the human Non Small Cell Lung Cancer (NSCLC) cell line PC-9 submitted to the cytostatic drug erlotinib. Here again, a division of the proliferating cell population into phases could be added, together with circadian control at phase transitions (another open modelling problem).

Another model [24], also relying on the McKendrick model, but more complex than the previously described ones, has been proposed to take into account both PK-PD models for the two main drugs in use in the clinic of colorectal cancer: 5-Fluorouracil (with added folinic acid, i.e., Leucovorin to potentialise it) and Oxaliplatin and the possibility of repair in cell populations that have been hit by cytotoxic drugs. Involving 3 phases (G_1, $S-G_2$ and M) and additional subpopulations R_1 and R_2, also structured in age and evolving in parallel with the first two, consisting of these cells that are under repair from cytotoxic insult by the two drugs illustrated on Fig. 5, it has also been used to solve once more the same therapeutic optimisation problem (minimising cancer cell population proliferation while maintaining the proliferation rate of the healthy cell population over a prescribed threshold).

In this model, circadian clocks also control phase transitions as in Section 5.2, but cytotoxic drugs are assumed to continuously exert their effects by sending cells to these parallel repair phases (a sort of 'delayed death': at any rate, these cells go out of the proliferating phases, but may come back to them at any moment), and not by blocking phase transitions. Then, since it is a linear model, the same optimisation principles used in Section 5.1 are used, minimising the proliferation rate of the cancer cell population while maintain that of the healthy cell population over a prescribed threshold, except that the optimised input functions are the flows of the two drugs in the general circulation, since the model includes a PK-PD representation of their fate in the organism from infusion until arrival on the proliferating cell population sites. The results, although good (see Ref. [24]), are less spectacular than in the previous model case, which again induces to speculate that the strongest drug effects on proliferation should occur on phase transitions, rather than on death rates,

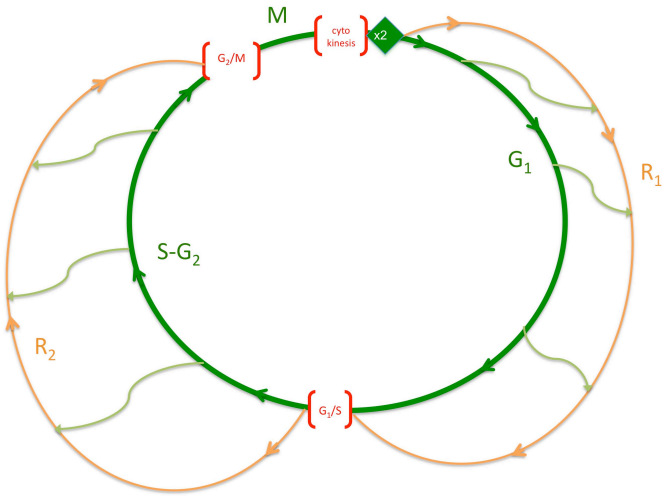

Fig. 5 From [24], illustration of a new model of the cell cycle with repair: cell cycle phases G_1 $(i = 1)$, $S - G_2$ $(i = 2)$ and M $(i = 3)$ with age-dependent variables, plus two additional subpopulations, R_1 and R_2 described by age-independent variables, to describe the fate of those cells that have been hit by drug-induced DNA damage and are waiting to be repaired - or sent to apoptosis. See Ref. [24] for details.

as has been assumed in this last model. On Fig. 6 is shown a theoretically optimal combination of the drugs 5Fluorouracil, Leucovorin and Oxaliplatin, another proof of concept of the method. Improving the PK-PD model and the representations of the modes of action of the drugs (possibly adding the representation of a cytostatic drug like Cetuximab), remain to be done to put it in realistic clinical settings.

Other models of the cell cycle in proliferating cell populations with control by circadian clocks have been published, firstly to establish likely mechanisms for such control, and then to propose optimal chronotherapeutic strategies with cytotoxic drugs, all in collaboration with Francis Lévi. In particular, Samuel Bernard and Hanspeter Herzel [13] used deterministic models with delays, while Attila Altinok and Albert Goldbeter [1, 2, 3, 4] used a clock-controlled cellular automaton model of the cell cycle to justify the chronotherapeutic strategies used by Francis Lévi in the clinic. Although these models do not propose optimisation algorithms for the drug delivery time schedules, they present interesting ideas from different points of view to guide the determination of such optimised regimens.

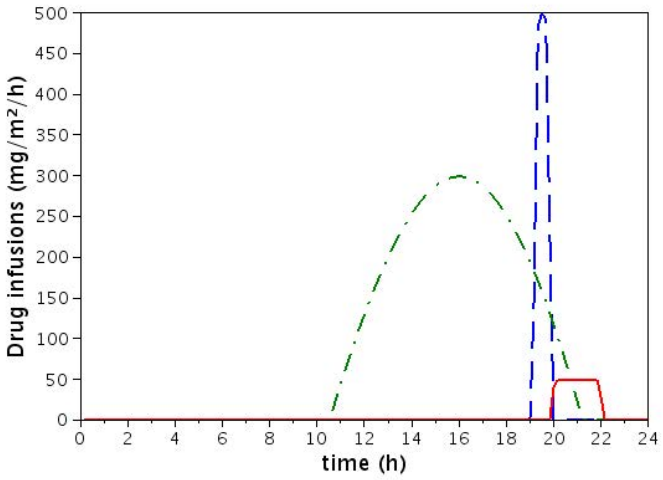

Fig. 6 From [24], illustration of an optimal infusion strategy proposing drug infusion flows on a 24-hour basis by a combination of Leucovorin (dash-dotted line), 5-Fluorouracil (dotted line) and Oxaliplatin (solid line), by infusions repeated every day in order to minimise proliferation of the cancer cell population while maintaining the growth rate of the healthy cell population above a prescribed toxicity threshold. See Ref. [24] for details.

6 Future prospects

6.1 Need for more knowledge on cell cycle control mechanisms

Now, to what extent are these theoretical models applicable in the clinic of cancers? Firstly, they are all based on the prediction of best drug delivery time schedules, and demand adapted technological appliances to put them in practice. While such devices are used in clinical chronotherapy, they should be adapted to preclinical trials on animals, which is not a simple problem - drug infusion in rodents, for instance, is usually performed only in boli, by lack of adapted devices -, not to mention the cost of programmable pumps, mundane limitations that so far have not given the possibility to experimentally test theoretically optimal drug delivery schedules.

Furthermore, much remains to be elicited in different fields of research related to chronotherapy: identification of PK-PD models and involvement of circadian control in them (a different model for each drug and each disease, to be further personalised according to patients' specificities, in particular gender [49]), identification of actual mechanisms of synchronisation between cells with respect to cell cycle timing, both in healthy and in cancer cell populations (is it true that cancer cell populations are poorly synchronised? what is the role of circadian clocks in cell syn-

chronisation? does there exist synchronisation mechanisms that rely on intercellular communication, e.g. using gap junctions?), identification of gating mechanisms at cell cycle phase transitions by Cyclin-Cdk complexes (shape of the gating function, with and without control by circadian clocks? this might be performed by FUCCI analysis, if one can find a fluorescent protein to be hybridised with activated Cdks), by p53 and other cell cycle fate determinant proteins, etc.

However, it is clear that before therapeutics with drugs should be called, with the complexity of their mechanisms of action needed to be represented, when the disease has reached a life-threatening level, preventive medicine must be more broadcast and popularised among healthy people (or supposed to be so), since cancers usually take a long time to develop in living organisms and can be more easily combated when taken at an early stage of their development. In this respect, disruptions of the circadian clock have been shown to enhance the development of tumours, experimentally in laboratory rodents [42, 43], but also by large epidemiological studies in humans [33, 62, 64, 92]. Conversely, re-establishing regular periodicity in daily rhythms by adapted (restricted) food intake regimens seems to go in the opposite and favourable direction in rodents [42, 105].

Both from the aforementioned commonsense and well established rules for a better quality of life (not always satisfactorily explained with respect to their mechanisms, but that nevertheless should be more widely broadcast), and from the aforementioned pending questions on molecular mechanisms involving circadian clocks, cell cycle determinants and anticancer drugs, one may see that the field of research in modelling for cancer chronotherapeutics is vast and has only begun to be explored.

6.2 Cancer chronotherapeutics and the immune system

In particular, modelling the immune response in cancer is still in its infancy. Pioneering models of the immune response, using immunotherapy associated with chemotherapy, have been published in recent years, in particular by Lisette de Pillis and Amy Radunskaya [34, 35, 36], and also by Peter Kim and colleagues [68, 80] and by Marcello Delitala and Tommaso Lorenzi [37], among others. However, none of them relates to an influence of circadian clocks, although it might exist, since it has been shown by Rune Smaaland and colleagues, using observations on samples from their own bone marrows, that DNA synthesis in bone marrow follows a circadian rhythm [96]. Furthermore, circadian rhythms of circulating lymphocytes have been evidenced in Man [75, 97].

Conversely, an influence of the immune response on the central circadian pacemaker is likely, since it has been observed that patients with high levels of circulating cytokines - that are emitted by immune cells surrounding the tumour, mainly T-lymphocytes - also show high degrees of fatigue and other even clearer signs of a disrupted central clock (ablated rhythms of blood cortisol and of rest-activity alternation) [87]. If such detrimental influence of the immune response on the clock is established, taking into account the tumour growth-enhancing effect of clock disrup-

tion mentioned in Section 2.4, this may mean that tumours use part of the immune response to their own advantage, a phenomenon to be potentially taken into account in immunotherapy models involving circadian biology.

If both the immune system and circadian clocks are to be taken into account in modelling for cancer chemotherapeutics, it should also be mentioned that some anticancer drugs exert a detrimental influence on circadian clocks [74, 100], and as regards the immune system, some of them may be detrimental to the immune system, and some may be beneficial to it, even so that their anti-tumour efficacy may be due to stimulation of the immune response [106, 107, 108].

6.3 Taking drug resistance into account

Is the other main pitfall of anticancer therapeutics, emergence of drug resistance, related to circadian clocks? ABC transporter activity [55] is one of the main mechanisms on which drug resistance relies, and it has been shown that some ABC transporters (P-gp and Abcc2) show circadian rhythms in their gene expression and protein concentration [5, 85]. Other mechanisms (e.g., enhanced activity of drug degradation enzymes), that show circadian rhythmicity, may also be responsible for the emergence of drug resistance in individual cells.

However, it does not seem that overcoming drug-induced drug resistance, which is a phenomenon occurring on a time scale that is not related with the 24-hour span - much longer in fact, due to mutations or epigenetic modifications, as mentioned in Section 3.2 - may benefit from chronotherapeutics. Mathematical models aiming at representing drug resistance, and optimisation methods of drug delivery have been proposed for some time already, distinguishing between a sensitive cell subpopulation and a resistant one [30, 31, 32, 51, 69, 99].

Quite recently, continuous models structured according to a phenotypic trait and based on integro-differential equations, that are common in ecology and are based on Darwinian selection principles, applied to the problem of emergence - and its overcoming by combinations of cytotoxic and cytostatic drugs - of drug resistant subpopulations in cancer cell populations, have been proposed [57, 71, 77]. They offer the advantage of allowing the possibility to represent slow evolution, according to the expression of a phenotype rather than by jumps due to pointwise mutations, of a cancer cell population, a feature which makes them amenable to represent epigenetic modifications on which drug-induced drug resistance is likely to rely when it is reversible. These models do not take into account so far the cell division cycle, since the drug targets are simply a proliferation rate and a death rate, without molecular support, but more complex models taking the cell cycle into account might be relevant to describe different molecular drug effects on proliferation and death.

How should one balance and put in perspective the two pitfalls of unwanted toxicity and of emergence of drug resistance for therapeutic applications? Most likely, the clinic will put a hierarchy between them, and this will depend on each cancer and each drug delivery problem. However, it is possible to take both problems si-

multaneously into account, as shown in [77]. In any case, it is not obvious how chronotherapeutics may be relevant in this perspective.

Conclusion

I have presented some mathematical models of cell population dynamics designed in the last ten years, aiming at optimising cancer chronotherapeutics. As long as the constraint chosen for the optimisation problem is the limitation of unwanted toxic side effects, proofs of concepts have been achieved, showing the interest of chronotherapeutics, even though many unknowns still remain to identify before such theoretical models may be applicable in the clinic, reinforcing those that are already in use. As regards the other main pitfall of cancer therapeutics, drug resistance, different cell population dynamics models, transposed from mathematical ecology and set at a different time scale and based on Darwinian selection principles, have begun to emerge, and so far chronotherapeutics has not proved relevant for them.

Acknowledgments

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